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Characteristics to Consider when Choosing an Animal Model for the Study of Lead Bioavailability

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Abstract

Most animal studies conducted to determine the bioavailability of lead have, in the past, employed rodents or lagomorphs as experimental models. In this paper issues and data are presented which raise questions and uncertainties about employing rodents or lagomorphs for investigations into the bioavailability of lead. These issues include: (1) the possible role of coprophagy and feeding behavior in reducing estimates of lead bioavailability; (2) anatomical and physiological differences related to coprophagy which may influence estimates of lead bioavailability derived in rats or rabbits; (3) evidence for relatively high biliary excretion of lead by rats and rabbits; (4) the possibility of a strong developmental component to the active transport of lead. The importance of addressing these and other questions in studies designed to determine the bioavailability of lead is discussed.

Introduction

Multimedia exposure of children to lead is recognised as an health problem of international proportions. Ingestion of soil and dust incidental to hand to mouth activity presents one of the principal direct pathways for exposure to non-dietary lead in areas with significant soil contamination. Environmental lead contamination derives from a variety of sources including lead based housepaint, auto emissions, smelter emissions, wind-blown tailings or mine wastes and mine waste deposits which have been used for residential development or have been redistributed as fill material in such areas.

Recently, a debate regarding the relative bioavailability of lead from different sources has developed (Steele *et al.*, 1989). In particular some indirect evidence has been interpreted to suggest that the bioavailability of lead from mining/milling operations is significantly less than that of lead from other sources. A preliminary review of issues pertaining to lead sources and their bioavailability has been presented by Chaney *et al.* (1988). As is evident from this review, a great deal of our present information on lead bioavailability is based on animal studies which used rodents as models. Regardless of the species employed, such studies are most informative if issues pertaining to particle size, metal speciation and chemical matrix are clearly addressed.

In response to the issues raised in the above-cited papers, a study of the site-specific bioavailability of lead in mill tailings has been conducted (LaVelle *et al.*, 1991). It became apparent during the study design phase that many issues relevant to the rat or rabbit models for lead bioavailability had been inadequately addressed in the literature. This paper will present a brief overview of these issues. It is hoped that the information will assist investigators in the design, conduct and interpretation of animal studies on bioavailability of ingested lead. While the material presented has particular importance for individuals interested in studies concerning the less soluble species of lead such as might be associated with mining, milling or smelting operations, much of the material presented is applicable to other forms of lead as well.

Definitions of Bioavailability

Definitions of bioavailability via the gastrointestinal tract or other routes may take different forms depending upon the laboratory procedures employed and the experimental intent of the investigator. Pharmacological definitions of bioavailability generally consider the area under the blood concentration *vs* time curve (AUC). Using this method, whole blood concentrations of the xenobiotic in question are plotted *vs* time following ingestion and are then compared with similar plots following intravenous administration. The ratio of AUC_{oral} to AUC_{iv} times 100 is then taken as a measure of percent absorption of the agent. An understanding of presystemic elimination (*i.e.* net excretion into the alimentary tract) in the animal model employed is important in interpreting estimates of bioavailability using the AUC technique. Thorough study of systemically delivered lead can provide information regarding transepithelial elimination into the alimentary tract. A potential limitation of the AUC technique is that it provides little information regarding non-linearities in the absorption *vs* time curve over the subchronic or chronic time frame.

Other definitions of bioavailability involve total mass balance where, for example, total chemical excreted in urine and feces and total retained in the body are measured. Such studies are most useful when absorption kinetics are considered. Steady-state blood levels reached after multiple dosing may be used as an indicator of bioavailability. Bioavailability might then be estimated as chemical in urine plus chemical retained in the body divided by the total chemical recovered. Again, knowledge of net excretion into the alimentary tract is essential to accurate estimation of the amount of chemical absorbed.

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Measures of bioavailability using AUC methodology should be distinguished from whole-body uptake of lead or other measures of cumulative body burden. Closely spaced measurements using the AUC methods can provide detailed kinetic information concerning absorption and elimination of lead from the blood. On the other hand, mass balance studies may provide some indication of accumulation of compounds such as lead after repetitive exposures. Coupled with tissue analysis for accumulated lead, mass balance studies augment our understanding of lead distribution. One area requiring further research concerns the kinetics of distribution and mechanisms involved with accumulation and release of lead from cortial and trabelcular bone. A thorough investigation of bioavailability might include both types of measures, especially for a toxicant such as lead which accumulates during chronic exposure.

Bioavailability at the level of the target cell is, of course, independent of metal species or matrix but may present particularly interesting and complex experimental challenges. Cellular investigations have shown that toxic metal ions may bind with and alter blood cell membrane structure and function (Weis and Haug, 1989). X-ray microprobe analysis has shown qualitatively that synaptosomal mitochondria may accumulate lead (Silbergeld *et al.*, 1977). Quantitative estimates of bioavailability at the level of the mitochondria would further our understanding of the mechanisms of lead neurotoxicity. *In vitro* work has shown that, while much circulating lead is found bound to erthrocyte membranes, these cells may be limited in their binding capacity (Barton *et al.*, 1980). This may have important implications for those interested in modelling the biokinetics of lead distribution and dose at the target organ or tissue. Due to the potential saturation of erthrocyte binding capacity, and the resulting nonlinearity of the whole blood to plasma ratio, care must be taken when interpreting bioavailability studies involving large doses of lead. For example, elimination of lead may be more rapid when large doses are administered with a concomitant reduction in the proportional dose retained at a target site such as liver, brain or bone.

Choice of the Animal Model

Toxicological data derived from animal studies is often used for the purpose of extrapolation to humans. Only rarely is human low-dose exposure data of adequate quality and quantity available for risk assessment purposes. In lieu of adequate human data, choice of an animal model is the initial and most crucial step in the conduct of experimental investigations for the purposes of understanding relevance to humans. All subsequent assumptions regarding data interpretation and extrapolation will rely upon the depth of understanding which the investigator has regarding the model employed and its physiological, pharmacokinetic and biochemical similarity to humans. The USEPA acknowledges the importance of the model choice for the purposes of extrapolation to humans (Barnes and Dourson, 1988).

> "Presented with data from several animal studies, the risk assessor first seeks to identify the animal model which is most relevant to humans, based on the most defensible biological rationale."

Some considerations to be addressed when choosing an animal model for studies of bioavailability of lead (Pb) will be presented in three categories. First, behavioral characteristics of the experimental animal model will be introduced. Secondly, anatomical considerations will be addressed. Finally, the importance of physiological and biochemical differences will be discussed with particular focus upon developmental changes which are especially critical when assessing the bioavailability of lead. It should be recognised that, while it is convenient for the purpose of this paper to address each of the above as separate aspects to be considered when choosing an animal model, none should be considered in isolation.

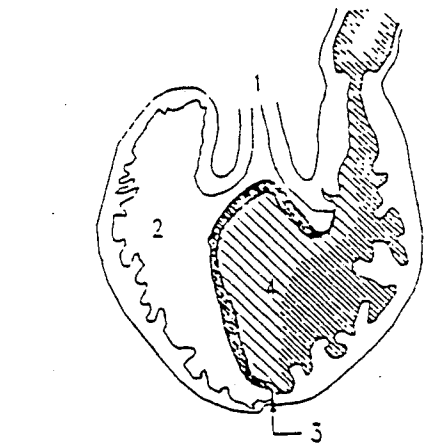
Behavioral considerations

Rodents such as mice and rats, which are commonly employed as models for extrapolation to humans, are altricial species attaining an ability to thermoregulate at approximately 20–25 days of age. Recent studies indicate that rodents may return to a state of thermal lability following exposure to heavy metals including lead (Watkinson and Gordon, 1990). In response to xenobiotic insult, rats employ both behavioral and physiological mechanisms to lower body core temperature thus attenuating both the absorption of xenobiotic and the toxic response while increasing potential for survival (Gordon, 1991).

Innate feeding behavior can greatly influence the bioavailability of lead. The presence of food in the stomach can clearly influence absorption of lead in humans (James *et al.*, 1985; Rabinowitz *et al.*, 1980). Rodents and lagomorphs are "continuous feeders" (Bivin *et al.*, 1979). Due to such continuous feeding habits the stomach of the healthy rabbit is never empty (Kraus *et al.*, 1984). Continuous feeding behaviour allows for maintenance of gastric floral growth required by the rodents and lagomorphs for digestion of cellulose and the release of essential nutrients and vitamins from plant material. It follows that both continuous feeding displayed by these experimental species and the presence of gastric flora may serve as a buffer for gastric fluid. Additionally, the presence of food and flora in the rodent or lagomorph stomach assures the continual presence of ligands for ionic lead in the form of negatively charged proteins, phytates and other phospholipid. Such adaptive behaviour and physiology by rodents may reduce the gastric dissolution of all forms of lead and other metals, hence greatly reducing measurements of metal bioavailability. By contrast, canines and swine, like humans, tend to ingest periodic 'meals' which are followed by gastric emptying. Complex regulation of gastric emptying by neural and humoral mechanisms assures that delivery of gastric contents to the duodenum does not exceed the body's capacity to emulsify and process these contents.

At this writing, estimates regarding the amounts of soil which children ingest and the times of day during which such events might occur are, at best, uncertain. It is likely that children are more exposed to environmental lead between, rather than during, meals. Modelling the maximal exposure which might reasonably be expected to occur by assessing bioavailability of lead-laden soil or other media on an empty stomach is only possible in species with periodic feeding behavior.

Rats and rabbits re-ingest fecal matter as an adaptive mechanism allowing for the digestion of cellulose and the absorption of essential nutrients and vitamins from plant



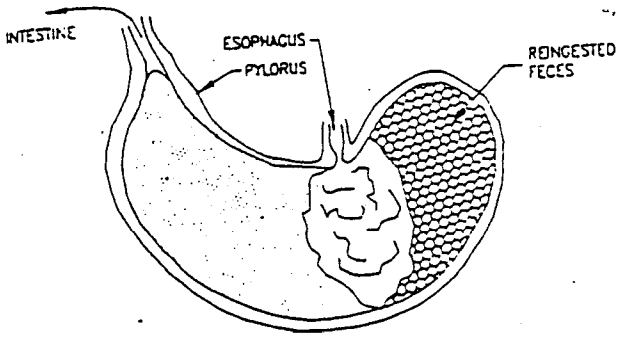
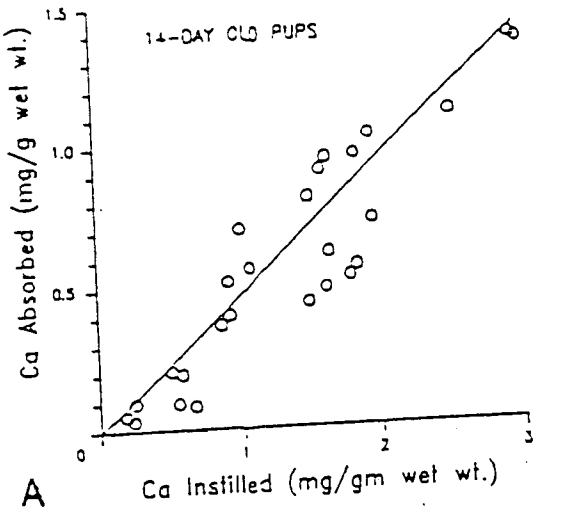
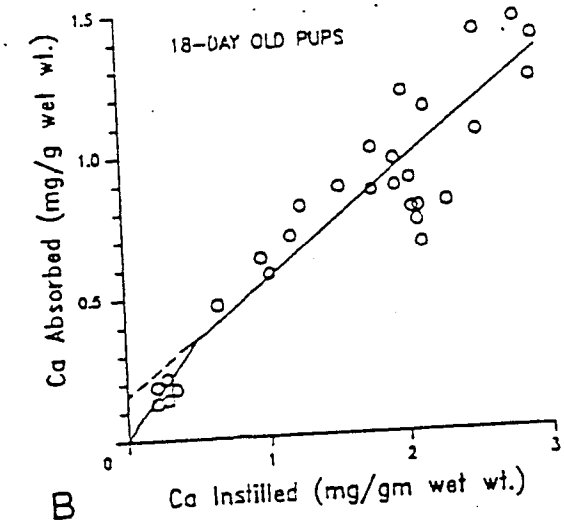


Figure 1 *Schematic representations of the rodent stomach: (a) Diagram of areas of the mucous membrane in the rat stomach: (1) cardiac region; (2) cutaneous (nonglandular) area; (3) line of transition from cutaneous to glandular mucous membrane; (4) cardiac glandular region (Hebel and Stromberg, 1976). (b) In the rabbit stomach ingested food is located in the pyloric region (1). Re-ingested fecal pellets are located in the large fundus (2) where they remain separated while fermentation proceeds.*





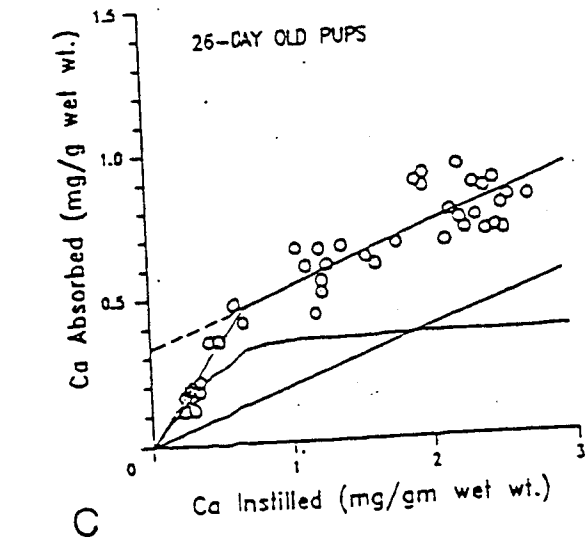


Figure 2 *Development of active calcium transport in the rat small intestine as measured using situ ligated duodenal loops. At 14 days of age (2a) calcium absorption occurs predominantly via passive diffusion across the brush border. At 18 days (2b) the initial development of active transport mechanisms is evidenced by curvilinear kinetics of the absorption curve at low dose and non-zero intercept at the ordinate. In the 26 day old rat (2c) both active (curvilinear) and passive (linear) components of the calcium transport are evident. From Dostal and Toverud (1984) with permission.*

material (Morot, 1911; Eden, 1940). This coprophagic behaviour displayed by rodents and lagomorphs creates problems for accurate determination of actual or relative measurements of bioavailability. Coprophagy introduces complications in the rodent model system since both essential nutrients and lead may be recycled (Thompson and Worden, 1956; Fullmer and Rosen, 1990). Without tedious and constant monitoring of the experimental rodent, the investigator can never be assured that measures of bioavailability are not biased by reingestion of previously excreted xenobiotic. Caging structures which deprive rodents from caprophagy may introduce uncertainties associated with mineral and vitamin

Table 1 *Comparison of the absolute and relative surface areas of the absorptive regions of the gastrointestinal tracts of humans and Rats. From DeSesso and Mavis (1989) with permission.*

Region	Human		Rat	
	Absolute surface area (m^2)	Relative surface area (region/body)	Absolute surface area (m^2)	Relative surface area (region/body)
Body	1.85	—	0.045	—
Stomach	0.0525	0.03	TBF[a]	TBF[a]
Small intestine	200	108	1.00	22
Duodenum	19[b]	10.3	0.08	1.8
Jejunum	138.6[b]	74.9	0.90	19.8
Ileum	42.4[c]	22.9	0.02	0.4

[a] TBF = To be found.
[b] Calculated using the data in Snyder *et al.* (1975), and proportion of mucosal surface area to length of intestine as 98:1.
[c] Calculated using the data in Snyder *et al.* (1975), and proportion of mucosal surface area to length as 20:1.

deficiency known to influence absorption of lead (Mahaffey-Six and Goyer, 1970). This experimental problem is greatly compounded by the rat's increased capacity for biliary excretion of lead discussed later in this paper.

Gastrointestinal anatomy and acid secretion

Discerning the role of gastric acidity in the bioavailability of various lead species is complex. Experimental background to fully understand the solution chemistry of metals in the stomach and anterior small intestine is not yet available. Active transport systems for calcium may also transport Pb across strong electrochemical gradients in the anterior small intestine shifting the solution chemistry far from equilibrium. As discussed above, the presence of gastric contents provides ligands for divalent metal ions, potentially influencing the bioavailability of lead across the gastrointestinal tract. For these reasons, equilibrium or pseudoequilibrium models of gastrointestinal solution chemistry are, at best, simplistic models with limited usefulness for the prediction of bioavailability. Experimental approaches to answering the question of the role of gastric contents might involve controlled comparisons of bioavailability in the presence and absence of gastric contents.

Gastrointestinal anatomy of the rodents has evolved to allow for digestion of plant material (Figure 1). Specialization of gastric anatomy to accommodate the digestion of plant material may be expected to influence dissolution of less soluble metal salts in the stomach such as those found in mining related waste. Figure 1a depicts a schematic tracing from a sagittal section through a rat stomach. Unlike the human or swine stomach, the rodent possesses a relatively large aglandular forestomach with rumen-like mucusal folds. The forestomach in both rats and rabbits is covered with a stratified squamous epithelium which serves primarily as a reservoir for gastric flora and reingested feces (Figure 1b). The forestomach of the rat and rabbit is devoid of acid secreting capacity. This is in sharp contrast to the human stomach which is predominantly glandular and devoid of indigenous flora. The acid secreting region of the rodent stomach is restricted to a smaller area anterior to the pylorus. While little empirical information is available regarding the interspecies differences in total acid secretion, such differences might play a significant role in lead bioavailability. Further research into this important area should be encouraged.

Acid secretion by human parietal cells is regulated by a variety of nervous and hormonal stimuli (Kutchai, 1983). Physiologically significant stimulants for acid secretion include acetylcholine, gastrin and histamine. Acetylcholine may be released by vagal activity or by intramucosal reflex acting directly on the parietal cell. Gastrin release is mediated by peptides or amino acids in the stomach. Distinct histamine receptors have been located on parietal cell membranes; however, the exact mechanism for histamine release is not clear. In all cases, stimulation of gastric acid secretion impinges upon the glandular portions of the stomach. In rodents the glandular regions of the stomach represent a relatively small portion of the overall glandular tissue in comparison with humans.

In general, four experimental approaches to determination of acid secreting capacity have been applied. Measurements of pH of gastric contents have been used to detect acid secretion capacity but this technique is unable to provide information about quantities of acid secreted over time or acid secretion set points (Garzon, 1982). Ussing chambers have been used as experimental techniques allowing for measurement of basal acid secretion and *in vitro* response to humoral stimuli (Duero *et al.*, 1981). Techniques involving gastric pylorus ligation (Ikezaki and Johnson, 1983) and continuous saline perfusion (Ackerman, 1982) have also been applied to the study of gastric acid secretion. These latter techniques introduce the undesirable experimental variables of reflex acid secretion and anethesia respectively.

Little comparative information regarding the total parietal cell mass in various experimental species is available. While acid output may be expected to be a function of parietal cell activity or cell density, it is the overall parietal mass that best relates to acid secretion during development (Yahav, 1989). Since gastric acid plays an important role in the initial solubilization of various lead species, animal models having parietal mass similar to humans would be the preferred choice

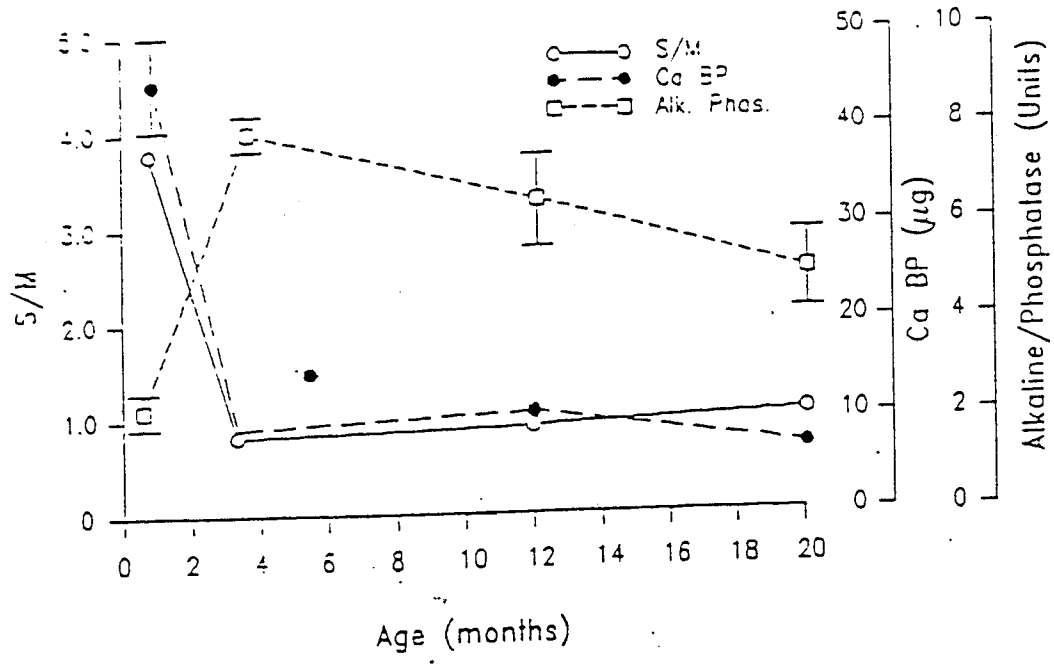


Figure 3 *Changes in duodenal calcium active transport, calbindin-D content and alkaline phosphatase as a function of age in Sprague-Dawley rats. Parameters were measured using everted duodenal sacs and active calcium transport is presented serosal/mucosal (S/M) ratio of radiolabeled calcium. From Armbrecht et al. (1979) with permission.*

for the conduct of metal bioavailability studies. This choice might be particularly important for the investigation of lead species soluble only under acidic conditions.

Active absorption of lead occurs at the anterior portions of the small intestine. Relative length of major subdivisions of the small intestine in rats and humans is presented in Table 1. Large differences in intestinal length among various experimental species may be expected to influence both active absorption of lead and enterohephatic circulation.

Development of absorption mechanisms

Calcium is thought to cross the intestinal brush border by a variety of energy-requiring and energy-independent mechanisms. Reviews of this subject have been presented elsewhere (Wasserman and Fullmer, 1983; Toverud, 1989). Several investigators have proposed that Pb may share a common transport process with calcium (Mayaffey-Six and Goyer, 1970; Smith *et al.*, 1981; Gruden, 1975; Barton *et al.*, 1978). These processes may involve: (1) transcellular routes which include the involvement of the calcium binding protein, calbindin-D (intestinal calcium binding protein) and are saturable at 2–5 mM calcium: (2) paracellular routes which occur at higher concentrations and are diffusion dependent displaying linear absorption kinetics and; (3) proposed vesicular transport mechanisms. Calcium binding proteins involved in the absorption of calcium across the gut may have a higher affinity for Pb (Fullmer *et al.*, 1985).

Comparative investigations into the ontogeny of the calcium transport process provide important insights into our understanding of lead bioavailability. Such comparisons are essential to both design and interpretation of bioavailability studies of lead. Much evidence exists to suggest a link between developmental stage and absorption of lead in humans (Ziegler *et al.*, 1978; Alexander *et al.*, 1973; USEPA, 1986). Mechanistic understanding of the age dependence of calcium absorption has been most thoroughly investigated in rats (Dostal and Toverud, 1984; Pansu *et al.*, 1983; Armbrecht *et al.*, 1979; Mooradian and Song, 1989). Figure 2 presents the progressive development of calcium absorption in the rat intestine. Active transport mechanisms for calcium in the gastrointestinal tract of the developing organism parallel increased calcium requirement for growth of the long bones and development of muscle and nervous tissue. It is evident that in the pre-weaned rodent, active transport plays a minimal role in the absorption of calcium (Figure 2a). Shortly post weaning, however, the maturity of the active transport process is evidenced by the bi-phasic nature of the dose *vs* absorption curve (Figure 2b). A distinction between active and passive mechanisms for calcium transport in the intestine are evidenced by the curvilinear (active) and linear (passive) components of the dose *vs* absorption curve. In the low dose range, active transport processes for calcium are dominant (Figure 2c).

Of great significance for the conduct of bioavailability studies for Pb is the abrupt termination of intestinal active transport processes for calcium at maturity (Figure 3) (Armbrecht *et al.*, 1979 with permission; Mooradian and Song, 1989; Armbrecht *et al.*, 1980). At maturity development slows. Epiphyseal plates are sealed. The calcium requirement diminishes, and the gastrointestinal transport mechanisms for calcium respond accordingly. Figure 4 presents the relative growth and development of swine, rats and humans. Sexual maturity in the rat occurs at approximately 7 weeks of age. This

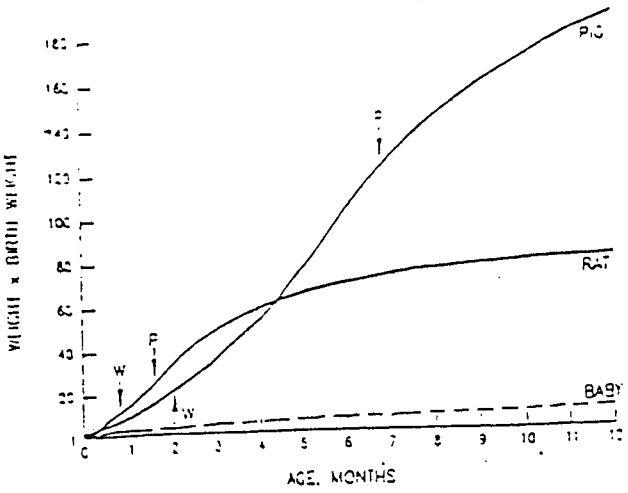


Figure 4 *Growth weight of rat, pig and human baby during the first year after birth. Birth weight = 1. From Widdowson (1968). W = Weaning; P = puberty.*

developmental milestone in rats occurs concurrently with cessation of active calcium transport. Assessment of bioavailability during or following the cessation of the active absorption component is inappropriate if an understanding of juvenile lead absorption is the intended purpose of the investigation.

The juvenile population is clearly defined as the population of most concern for exposure to environmental lead. It is likely that juvenile environmental exposure to Pb occurs in the low-dose range where active transport dominates the absorption process. If one presumes that Pb and Ca transport follow similar absorption kinetics, as the available evidence would strongly suggest, conduct of bioavailability studies for lead must be conducted on juvenile organisms.

The role of bile secretion

Fecal excretion of Pb via bile secretion and enterohepatic circulation can vary widely among various experimental species. Species differences in biliary handling of Pb may greatly influence measures of absolute or relative bioavailability. Comparative investigations of biliary excretion of lead in rats, rabbits and dogs have been conducted by Klaasen and Shoeman (1975). These investigations found profound species differences in the rates of biliary excretion of lead. Rabbits were found to excrete Pb via the biliary route at rates approximately one-half that of rats, while dogs displayed biliary excretion rates less then one-fiftieth that of the rat. Important physiological differences in biliary excretion have also been identified (Erlinger, 1987). The bile ducts alter the volume and composition of the bile fluid prior to entry into the digestive tract. Contribution of the bile ducts to overall bile flow is significant in the canines and primates but much smaller in rabbits, rats and guinea pigs. Bile acid transport in rats appears to be a saturable carrier-mediated process (Stremmel and Berk, 1986). Investigations into the bioavailability of Pb should consider the role of biliary excretion and enterohepatic circulation from a comparative perspective if sound estimates of bioavailability of lead in humans is the goal of the study.

Summary

The issue of lead bioavailability remains an important barrier to an understanding of childhood exposure to this environmental hazard. The international pervasiveness of the problem, the sensitivity of the juvenile population and the apparent persistence of neurologic endpoints of lead toxicity all contribute to the need for reliable estimates of lead bioavailability. In establishing estimates of lead bioavailability, toxicologists are obligated to apply all available information regarding the comparative behavior, anatomy, physiology and pharmacokinetics of the experimental model being employed. Much of the information available regarding the molecular mechanisms of lead absorption and toxicology has been derived from studies conducted using rodents. Some investigators have questioned the use of rodents for the purpose of understanding the molecular aspects of lead and calcium metabolism due to uncertainties regarding the extrapolation to humans (Fullmer and Rosen, 1990). Other investigators continue to employ rodents for the purpose of understanding the absorption and distribution of lead (Killinger, 1990).

Cost and difficulty in handling are clearly recognised as real world constraints to the conduct of animal research. Regardless of the species employed to assess the biokinetics of lead absorption and distribution, a comprehensive assessment of the model being employed and its relevance to humans must be incorporated into estimates of bioavailability. Without such an assessment, missrepresentations and missunderstandings of the bioavailability of lead will be risked.

We believe that the weight of evidence suggests that studies of metal bioavailability, particularly lead bioavailability studies, conducted in rodents or lagomorphs should be viewed with caution. Evidence presented in this chapter which justifies caution in the interpretation of bioavailability studies conducted in rats and rabbits includes: (1) coprophagic and continuous feeding behaviour of these model species; (2) difficulties in assessing important developmental aspects of lead bioavailability in rats and rabbits; (3) evidence for relatively high rates of bile excretion of lead in rats and; (4) profound anatomical differences between these species and humans. Better model species are readily available for the study of metal bioavailability. More complete characterization of these alternative models should be encouraged.

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